

REMARKS

Claims 1-3, and 9 have been cancelled without prejudice or disclaimer of their later prosecution in one or more applications. Claims 4-8, 10, and 11 are pending and considered. Applicants propose to amend claims 4 and 5 as set forth above.

Claims 4 and 5 have been amended to recite “and containing neither an F-spondin domain nor a repeat site.” Claim 5 has also been amended to recite “wherein the encoded polypeptide contains neither an F-spondin domain nor a repeat site.” Support for claims 4 and 5 can be found in original claims 4 and 5 and throughout the specification, for example, at page 4, lines 23-25.

Claim 5 has also been amended to correct certain informalities. Specifically, claim 5 has been amended to correct the spelling of “polynucleotide,” to eliminate “the group consisting of,” and to delete an extraneous “of.”

Thus, the claims are fully supported by the application as originally filed and the amendment adds no new matter. With entry of this Amendment, claims 4-8, 10, and 11 are pending for examination.

REJECTION BASED ON RESTRICTION REQUIREMENT

At page 2 of the *Action*, the Examiner stated that “[t]his application contains claims 1-3 and 9 drawn to an invention nonelected with traverse...[a] complete reply to the final rejection must include cancellation of nonelected claims or other appropriate action,” citing to the Manual of Patent Examining Procedure Eighth Edition (August 2001) (“MPEP”) § 821.01. Applicants have cancelled claims 1-3 and 9, and thus the Examiner’s rejection is moot.

Applicants respectfully request reconsideration and withdrawal of the rejection based on MPEP § 821.01.

CLAIM OBJECTIONS

At page 12 of the *Action*, the Examiner objected to claim 5 for recitation of the language “that of binds.” Applicants have amended claim 5 to delete the word “of.” Thus, the Examiner’s objection is moot.

REJECTIONS UNDER 35 U.S.C. § 112, FIRST PARAGRAPH

Enablement

At page 3 of the *Action*, the Examiner maintained the rejection of claims 4-8, 10, and 11 under 35 U.S.C. § 112, first paragraph, alleging that, “the specification, while being enabling for the polynucleotides of SEQ ID NO:1, does not reasonably provide enablement for polynucleotides of alternative sequence as claimed including deletion, insertion, substitution and degenerate sequences.” *Final Office Action* at page 3.

According to the Examiner, the “skilled artisan also recognizes that immunological responses depend upon the structural characteristics (conformation) of the particular protein (amino acid sequence) targeted and that variations in sequence may effect such structure and immunological recognition.” *Id.* at pages 3-4. The Examiner then noted that certain claims recite “deletion, insertion, and substitution mutations as well as degenerate sequences,” and are thus “directed to polynucleotides encoding peptides with greater than single amino acid substitutions, deletions and insertions and to partial peptide fragments which bind CR-50 antibody.” *Id.* at page 4. The Examiner stated that “there is no disclosure of those residues which may be replaced, modified or deleted without abrogating the disclosed immunological reactivity.” *Id.*

Applicants respectfully traverse. In the Amendment dated April 14, 2003, Applicants argued that it is not necessary that the specification enable one skilled in the art to predict

precisely which changes in a polypeptide will not affect activity, because modifying a polypeptide and testing it for activity does not involve undue experimentation, citing *In re Wands*. See Amendment of April 14, 2003 at pages 8-12; 858 F.3d 731 (Fed. Cir. 1988). Similarly, Applicants argued that the specification enables both determinations of which compositions containing the claimed molecules are capable of stimulating the assembly of Reelin molecules and diseases that are capable of being treated by these claimed compounds. *Id.* The Examiner, however, argued that “[t]he ability to “make and test” is not the standard for an enabling disclosure,” and that the instant specification fails to identify that structure which is required for the claimed biological activities of’ the claimed invention. *Final Office Action* at page 7. The Examiner argues that “[i]n the absence of guidance, a practitioner of the art of molecular biology would have to resort to a substantial amount of experimental trial and error to produce the peptides as claimed.” *Id.*

The Examiner has argued, essentially, that protein chemistry is an unpredictable field, that without specific information about which residues within the CR-50 region are critical for binding of the CR-50 antibody, making a peptide with the claimed properties is a matter of trial and error. However, the Examiner overlooks an express element of the claims. The peptides encoded by the claimed polynucleotides **bind to the CR-50 antibody**.

Screening assays to measure the ability of an antibody to bind to a specific protein are well known in the art. In fact, the Federal Circuit recognized antibody screening assays as routine in the art as early as 1986. See *Hybritech, supra*. The Court in *Hybritech* stated that “screening methods used to identify the necessary characteristics, including affinity of the monoclonal antibodies used in the invention were **known in the art**.” (emphasis added). *Id.* at 1384. The opinion itself describes a number of immunoassays, including a competitive

immunoassay and a sandwich assay. *Id.* at 1369-70. Furthermore, Applicants also teach a cell adhesion assay to measure antibody binding to the Reelin peptides. *See* specification at Example 3.

It is true that the claims are drawn to polynucleotides encoding different CR-50 region-containing polypeptides and not to the CR-50 monoclonal antibodies themselves. A screening assay used to measure the binding of a monoclonal antibody to a peptide, however, can also be used to measure the binding of a peptide to a monoclonal antibody. If, as the Federal Circuit has stated in *Hybritech*, it is routine in the art to screen monoclonal antibodies for binding to an antigen, it must also be routine in the art to screen antigens (e.g., polypeptides) for binding to a monoclonal antibody. Here we have an identified monoclonal antibody (the CR-50 antibody) and a Reelin epitope (the CR-50 region) that is taught by Applicants to be the specific binding site for that monoclonal antibody. One of ordinary skill in the art should be readily able to screen a panel of polypeptides having various CR-50 permutations in a CR-50 monoclonal antibody (the one identified in the specification or one generated by others) binding assay and rapidly determine which have the claimed ability to bind to the CR-50 monoclonal antibody. Moreover, all of this can be done without undue experimentation.

There is no need for the specification to provide even greater description of the CR-50 region than it already has, because permutations of the region can be readily assessed as to whether or not they meet the claim limitations. No undue experimentation is required to practice the claimed invention, and the claims are fully enabled by the specification and the art. Applicants respectfully request reconsideration and withdrawal of this rejection.

New Matter Rejection

At page 12 of the *Action*, the Examiner rejected claims 4-8 and 10-11 under 35 U.S.C. § 112, first paragraph, alleging that the claims contain new matter. Specifically, the Examiner states that the claims “appear to optionally include a F-spondin domain or a repeat site as indicated by the or (alternative language) which differs from the previous negative limitations of not (neither) a F-spondin domain nor a repeat site.” *Final Office Action* at page 12-13. Solely to expedite prosecution and without acquiescing to the Examiner’s contentions, Applicants have amended claims 4 and 5 to more clearly indicate that the claimed polynucleotides encode polypeptides that contain **neither** an F-spondin domain **nor** a repeat site. Claims 6-8, 10 and 11 depend on claims 4 and 5 and thus also encompass this limitation. Therefore, the Examiner’s new matter rejection is moot.

Public Availability of Antibody

At page 13 of the *Action*, the Examiner also rejected claims 4-8 and 10-11 under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the enablement requirement. The Examiner stated that “[w]ith respect to the amended claims requiring antibody CR-50 epitope structure and binding, the specification is non-enabling because there is insufficient assurance that the antibody is publicly available.” *Final Office Action* at page 13. The Examiner stated that

[t]he specification lacks deposit information for the deposit of antibody CR-50. Because it is not clear that the antibody is definitively known and publicly available or can be reproducibly isolated from nature without undue experimentation and because the claims require the use of CR-50, a suitable deposit for patent purposes is required. *Id.*

Applicants respectfully traverse.

According to MPEP 2404.02, “Applicant may show that a deposit is not necessary even though specific biological materials are required to practice the invention if those biological materials can be made or isolated without undue experimentation.” Here, Applicants’ specification teaches the nucleotide and polypeptide sequence (*see, e.g.*, SEQ ID NO: 1 and 2) of the specific Reelin region which is recognized by the CR-50 monoclonal antibody. One of ordinary skill in the art would be readily able to prepare an antibody that recognizes this epitope using standard molecular biological techniques. In fact, courts have expressly recognized that the process of making monoclonal antibodies is well known in the art. The hybridoma technique,¹ for example, was described in a 1986 Federal Circuit opinion. *See Hybritech Inc. v. Monoclonal Antibodies, Inc.* 802 F.2d 1367, 1369 (Fed. Cir. 1986). In the intervening 14 years between this decision and the priority date for the instant application, this and other techniques for monoclonal antibody production have only become increasingly routine in the art. The CR-50 monoclonal antibody can be readily reproducibly isolated from nature, and its manufacture does not require undue experimentation. A deposit, therefore, is unnecessary under MPEP § 2404.02.

Applicants respectfully request reconsideration and withdrawal of this rejection.

Possession of the Claimed Invention

At page 15 of the *Action*, the Examiner also rejected claims 4-8 and 10-11 under 35 U.S.C. § 112, first paragraph, as allegedly “containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the

¹In the hybridoma technique, mice are immunized with the antigen of interest (here, the CR-50 epitope from Reelin), and hybridomas producing the monoclonal antibody of interest are created by fusing spleen cells from these mice to myeloma cells. When cultured, the hybridomas produce monoclonal antibodies against the antigen of interest (here, the CR-50 antibody).

inventor(s), at the time the application was filed, had possession of the claimed invention.” *Final Office Action* at page 15. Specifically, the Examiner stated that

the claims as written include polypeptides comprising CR-50 antibody recognition sites of Reelin protein and either a F-spondin domain or a repeat site, but not both. Additionally the claims recite compositions binding antibody CR-50 and that stimulate the assembly of Reelin molecules. However, the instant disclosure of a single polypeptide, that of SEQ ID NO: 2, does not adequately support the scope of the claimed genus, which encompasses a substantial variety of subgenera. *Id.*

Applicants respectfully traverse.

First, Applicants have amended claims 4 and 5 to make clear that the claimed polynucleotides encode polypeptides that lack both an F-spondin domain and a repeat region. Thus, the variety of subgenera encompassed by the claimed genus is expressly set forth in the specification. *See* specification at page 4, lines 23-25.

Second, as the Examiner observed, “a description of a genus of cDNAs may be achieved by means of...**a recitation of structural features common to the genus, which features constitute a substantial portion of the genus.**” (emphasis added) *Id.* at page 16. Both claims 4 and 5 (and thus claims 6-8, 10, and 11, which depend therefrom) refer specifically to the CR-50 antibody recognition site of Reelin protein and require that the encoded polypeptides bind to the CR-50 antibody. The genus claimed by Applicants, therefore, is that of polynucleotides encoding polypeptides comprising a CR-50 antibody recognition site of Reelin protein (or deletions, substitutions, additions thereto, or degenerates thereof), where the polypeptide binds to the CR-50 antibody, and where the polypeptide does not contain an F-spondin domain or a repeat region. Applicants’ specification teaches the structural features common to this genus, namely the sequence of the CR-50 recognition site, and that the epitope is responsible for

binding of the CR-50 antibody to Reelin. *See* SEQ ID NOS: 1 and 2, and specification page 4, lines 11-12 and Example 3.

Therefore, Applicants teach the requisite structural features of the claimed genus in a manner that conveys to one skilled in the relevant art that the inventors were in possession of the claimed invention at the time the application was filed. Applicants respectfully request that the Examiner reconsider and withdraw the instant rejection.

Applicants respectfully request reconsideration and withdrawal of each of the rejections under 35 U.S.C. § 112, first paragraph.

REJECTIONS UNDER 35 U.S.C. § 112, SECOND PARAGRAPH

At page 17 of the *Action*, claims 4-8, 10, and 11 were rejected under 35 U.S.C. § 112, second paragraph, for allegedly “failing to particularly point out and distinctly claim the subject matter which the applicant regards as the invention.” *Final Office Action* at page 17.

Specifically, the Examiner stated that:

[w]hile the art...generally teaches such regions and proteins, the references fail to teach the exact residues that correspond to the regions. Thus, applicant's inexact reference to the residues or regions encompassed or excluded by recitations and traversal of the relevant prior art, fails to establish the requisite metes and bounds of the terms within the claim. *Id.*

Applicants respectfully traverse. The existence of the F-spondin domain and the repeat region in the Reelin protein was known in the art at the time of the filing of the instant application, as were the residues corresponding to these domains. First, the amino acid and nucleotide sequences of Reelin and F-spondin were known at the time of filing of the instant application. *See* specification at page 2, lines 9-14. Second, as Figure 1 of de Bergeyck et al., *J. Neurosci Methods*, 82:17-24, 1998 (cited by the Examiner) shows, the regions of Reelin with F-spondin homology and the characteristic repeats had been identified at the time of filing of the instant

application. While the two references cited by the Examiner may not have specifically identified the residues corresponding to these domains by number, one of ordinary skill in the art would have been readily able to identify the particular residues involved based on the described characteristics of these two regions. Finally, the art has recognized specific amino acid residues corresponding to these regions, as demonstrated by Figure 4 of Tissir et al., *Nat. Rev. Neurosci.*, 4:496-505 (2003) (copy enclosed). Applicants therefore submit that the Reelin F-spondin domain and repeat region were well-defined in the art at the time of the filing of the instant application and need no further delineation by Applicants.

The Examiner also stated that “the terminology not...or is indefinite as to whether one or both of the elements is to be absent.” *Id.* As previously discussed, Applicants have amended claims 4 and 5 to more specifically point out that the claimed polynucleotides encode polypeptides lacking both an F-spondin domain and a repeat region. Thus, the Examiner’s rejection on this ground is moot.

Accordingly, Applicants respectfully request reconsideration and withdrawal of the enablement rejection under 35 U.S.C. § 112, second paragraph.

REJECTIONS UNDER 35 U.S.C. § 102

Rejection Under 35 U.S.C. § 102(b)

At page 18 of the *Action*, the Examiner rejected claims 4-8, 10, and 11, under 35 U.S.C. § 102(b) as allegedly being anticipated by de Bergeyck et al., *J. Neurosci. Methods* 82:17-24, 1988. *Final Office Action* at page 18. Applicants respectfully traverse this rejection.

The Examiner stated that “Peptide H164-496 is noted to be deleted in both the F-spondin region as well as the C-terminal repeat region domains. As the peptide comprises residues 230-346, the CR-50 epitope region it would bind the CR-50 antibody and the polynucleotides

encoding the same residues shares similarity to SEQ ID NO:1.” *Id.* The Examiner, however, is incorrect. Peptide H164-496 is missing only a portion of the F-spondin domain. As described in Figure 4 of Tissir et al., *Nat. Rev. Neurosci.*, 4:496-505 (2003) (copy enclosed), the F-spondin domain corresponds to amino acids 28-190 of Reelin. Peptide H164-496 includes amino acids 164-496 of Reelin, and therefore encompasses 27 amino acids of the F-spondin domain. Claims 4 and 5 (and therefore claims 6-8, 10, and 11, which depend therefrom) recite that the polypeptide encoded by the claimed polynucleotide does **not** contain an F-spondin domain.

For a reference to anticipate it must disclose every element of the claim. *See Hybritech* 802 F.2d at 1379. Because de Bergeyck does not disclose every element of the claimed invention, it cannot anticipate claims 4-8, 10, and 11.

Rejection Under 35 U.S.C. § 102(e)

At page 10 of the *Action*, the Examiner maintained the rejection of claims 4-8, 10, and 11, under 35 U.S.C. § 102(e) as allegedly being anticipated by Curran et al., US 6,323,177. *Final Office Action* at page 10. Applicants respectfully traverse this rejection.

The Examiner observed that Curran teaches the full-length Reelin polypeptide, “sharing 100% similarity with instant SEQ ID NO: 1, residues 1-351 and encoding SEQ ID NO:2, residues 1-117.” *Id.* The Examiner further stated that Curran teaches “isolated nucleic acids inclusive of various insertion, deletion and addition mutants, as well as fragments thereof encoding particular polypeptides. These mutants include N'- and C'-terminal deletions as recited in column 20, lines 4-14 and column 22, line 48-column 23, line 7.” *Id.* at pages 10-11. The Examiner went on to identify teachings in Curran of vector constructs, host cells, fusion proteins, methods of producing polypeptide, compositions comprising polynucleotides, and therapies and methods of treatment. *Id.* at page 11.

In Applicants' Amendment filed April 14, 2003, Applicants argued that for a reference to anticipate it must disclose every element of the claim. *See Hybritech*, 802 F.2d at 1379. Further, negative limitations are permitted. *See MPEP* § 2173.05(i). Applicants argued that because claims 4 and 5 (and thus also dependent claims 6-8, 10, and 11) each recite the negative limitation that the encoded polypeptide not contain either of an F-spondin domain and a repeat site, and Curran does not teach truncated Reelin peptides that lack both the F-spondin domain and the repeat site, Curran cannot anticipate the claimed invention. *See Amendment of April 14, 2003* at page 13.

The Examiner found these arguments unpersuasive, stating that

[t]he Curran reference teaches various peptide modifications as disclosed including regions that are N' and C' terminal truncations and which represent epitope structures of the CR-50 antibody. While applicant's claims do not delimit the residues which correspond to the invention the art and specification clearly establish that the F-spondin domain is within the N'terminus and the repeat region is within the C'terminus. *Final Office Action* at page 11-12.

This characterization, however, is incorrect. As shown in Figure 1 of de Bergecyk et al., *supra*, and Figure 4 of Tissir et al., *supra*, the Reelin repeat region is **not** C-terminal. Rather, it spans residues 501-3428 of the Reelin protein, followed by a C-terminal stretch of 33 amino acids rich in basic residues. *Id.* Curran itself states: "At the N terminus, Reelin contains a cleavable signal peptide and a small region of similarity with F-spondin....At the C terminus of Reelin there is a stretch of positively charged amino acids. The main body of the protein comprises a series of either internal repeats of 350-390 amino acids...." Col. 19, lines 2-8. Thus, even Curran itself teaches that the repeat region is not C-terminal, but rather in the "main body of the protein." *Id.* The truncations taught by Curran therefore do not encompass the claimed sequences, which lack the F-spondin domain and the repeat region. Because Curran does not disclose every element of the claimed invention, it cannot anticipate claims 4-8, 10, and 11.

Accordingly, Applicants respectfully request reconsideration and withdrawal of the anticipation rejections under 35 U.S.C. § 102.

CONCLUSION

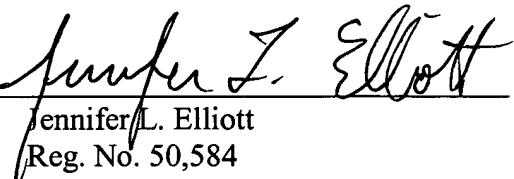
In view of the foregoing amendments and remarks, Applicants respectfully request the reconsideration and reexamination of this application and the timely allowance of the pending claims. If the Examiner does not consider the application to be in condition for allowance, Applicants request that the Examiner call the undersigned ((650) 849-6611) to arrange an interview prior to taking action.

Please grant any extensions of time required to enter this response and charge any additional required fees to our Deposit Account No. 06-0916.

Respectfully submitted,

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REELIN AND BRAIN DEVELOPMENT

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Over the last 50 years, the *reeler* mutant mouse has become an important model for studying normal and abnormal development in the cerebral cortex and other regions of the brain.

However, we are only just beginning to understand the actions of reelin — the protein that is affected by the *reeler* mutation — at the molecular and cellular level. This review discusses the most recent advances in this research field, and considers the merits of the various models that have been put forward to explain how reelin works.

LISSENCEPHALY
Literally meaning 'smooth brain'. Lissencephaly is a human brain disorder that is characterized by absence or reduction of the cerebral convolutions.

VENTRICULAR ZONE
The proliferative inner layer of the developing brain and spinal cord.

SUBVENTRICULAR ZONE
A layer of cells in the developing brain that is generated by the migration of neuroblasts from the adjoining ventricular zone.

RHOMBIC LIP
A specialized germinal matrix located at the posterior edge of the cerebellar anlage that gives rise to the granule cells of the cerebellum.

Following their description more than 50 years ago¹, *reeler* mice were the only known animal mutants with malformations of the cerebral cortex. Understandably, they generated a lot of interest, which increased further after the cloning of the affected gene — *reelin* (*Reelin*)². As the *reeler* mouse model has been exhaustively reviewed³, and the Reelin signalling pathway has recently been discussed⁴, we will not attempt to cover the field extensively, but instead will focus on recent findings that concern the role of Reelin during brain development. Our understanding of the action of Reelin remains sketchy, and we will try to point out caveats as well as advances in this field. After a presentation of the *reeler* malformation, we will summarize present views on the biochemistry of Reelin signalling, and discuss observations and hypotheses about the putative mechanism of action of this interesting protein.

***Reeler* and *reeler*-like phenotypes**

In rodents, Reelin deficiency results in the *reeler* phenotype, several alleles of which have been described in mice and rats^{3,5}. In humans, it is the cause of the Norman-Roberts type of LISSENCEPHALY⁶. The human phenotype is only known from medical imaging, but it seems to be similar to its murine counterpart. In mice, mutations of Disabled 1 (Dab1)⁷⁻⁹ and double mutations of two lipoprotein receptors, very-low-density lipoprotein receptor (VLDLR) and apolipoprotein E receptor type 2 (ApoER2), generate similar phenotypes that will be referred to as *reeler*-like throughout this review^{10,11}.

In *reeler* and *reeler*-like mice, neurons are generated in germinative zones (VENTRICULAR ZONE, SUBVENTRICULAR ZONE and RHOMBIC LIP) as in wild-type animals, and their

migration is initially normal. However, as they approach their destination, *reeler* neurons fail to form normal architectonic structures. This is illustrated in FIGS 1 and 2 for the embryonic cortex, and in FIG. 3 for the cerebellum and inferior olfactory complex. It seems that there is an instruction at the end of migration that is lacking in *reeler* mutants, so that neurons do not recognize their proper location and orientation at the end of their migration pathway. The rest of the differentiation program is unaffected — normal neuronal classes are formed, dendritic trees and axons ramify and connect almost normally with their physiological targets, and gliogenesis and myelination are not directly altered. However, due to abnormal neuronal positioning, the dendritic trees and initial axonal pathways are often distorted. The defect is most severe in the cerebral cortex, hippocampus and cerebellar cortex, but subtle anomalies have been identified at every location that has been searched, including the inferior olive, olfactory bulb, cochlear nuclei, facial nerve nucleus, thalamus and tectum (reviewed in REF. 3). Defects were also recently demonstrated in the retina¹² and spinal cord¹³.

Reelin, the protein that is defective in *reeler* mice, is secreted by some neurons, such as Cajal-Retzius cells in the cortical marginal zones and cerebellar granule cells, and it acts through the extracellular milieu on neighbouring target cells — cortical plate cells and Purkinje cells, respectively — to provide an architectonic signal. Reception of the Reelin signal requires the presence of at least one of two receptors of the lipoprotein receptor family on the surface of target cells, namely VLDLR and ApoER2. The signal is then transduced by tyrosine phosphorylation of the intracellular adaptor Dab1. In some

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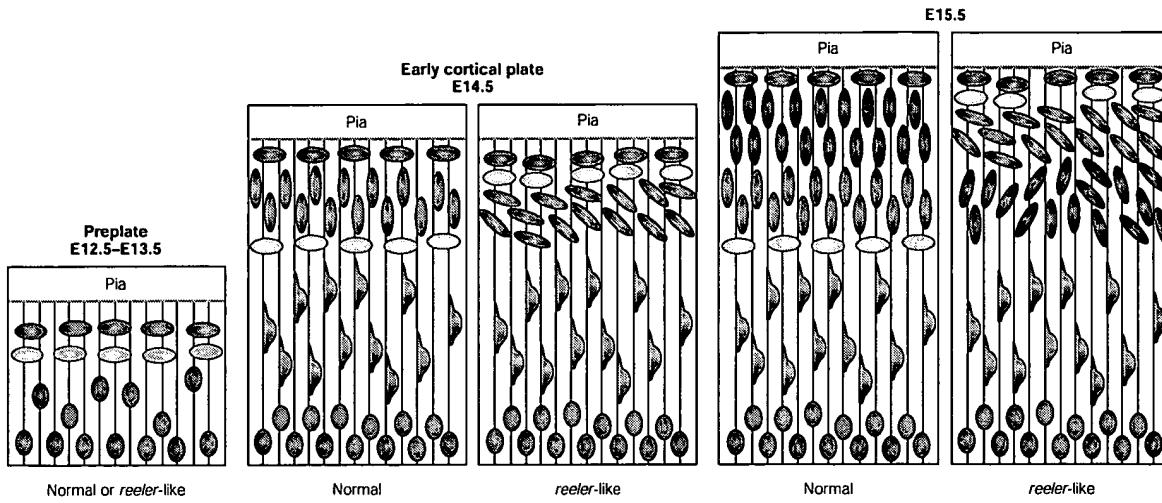


Figure 1 | Schematic view of early cortical development in mice. At embryonic day (E) 13.5 (the preplate stage) pioneer neurons migrate — probably by somal translocation¹⁶ — and form a loose horizontal network. Some are reelin (Reln)-positive (red) and others are Reln-negative (pink). At this stage, the *reeler* phenotype is not evident. At E14.5, as migratory distances and the complexity of the environment increase, cells migrate along radial glial fibres. The first cohort of cells condenses in the cortical plate (green), splitting the preplate into two contingents. The *reeler* cortical plate is populated with cells in an oblique orientation and does not split the preplate into two components. At E15.5, a second cohort of cells (blue) migrates through the normal cortical plate and settles superficially, forming an inside to outside gradient. In *reeler* mutants, the second cohort settles in the deep tiers of the cortical plate, forming an outside to inside gradient.

locations, such as the cerebral cortex, hippocampus, cerebellum and inferior olive, Reln-producing and Reln target cells are spatially segregated. In other regions, such as the retina and spinal cord, the source and target cells are close to each other or even mixed. In a few cases, for example in human Cajal–Retzius cells, the same cells are positive for Reln and Dab1, implying that there is an autocrine loop^{14,15}. Intriguingly, the *reeler* phenotype is most evident when the source and target cells are segregated, and this might be relevant to the mechanism of action of Reln. In addition to the brain, Reln is also expressed at lower levels in peripheral organs, such as the liver¹⁶, kidney and a few others^{17–19}. However, the function of the pathway in non-neuronal tissues is still largely unexplored.

Reln and its partners

The *Reln* gene is about 450 kb long and maps to mouse chromosome 5 and human 7q22 (REFS 20–22). It is similar in mouse, man and probably other species. *Reln* contains huge introns — in particular, introns one, two and three, which are 57, 59 and 54 kb long, respectively, in the mouse, and even larger in man. So far, almost nothing is known about the regulation of *Reln* mRNA expression. The proximal promoter is CG-rich and contains no TATA box. Its methylation pattern has received some attention recently^{23–25}, but there is still considerable scope for further investigation. This promoter has significant activity in reporter systems, but is probably not sufficient to account for the fine regulation of *Reln* mRNA expression in neurons such as the Cajal–Retzius cells. 5'RACE analysis from exon 2 did not show any additional promoter

(I. Bar and A.G., unpublished data), and the genomic region upstream of the *Reln* gene contains mostly repeated sequences that lack specific features. This implies that regulatory elements are located in introns, but this remains to be analyzed. A better definition of the transcriptional control of *Reln* will be required if we want to gain a better understanding of the roles of Reln and Cajal–Retzius cells in cortical development and evolution²⁶.

The *Reln* mRNA is about 12 kb in length and consists of 65 exons²². Two ALTERNATIVE SPLICING events have been found²⁷: a micro-exon of 6 nucleotides (exon 64, which encodes Val–Ser) is included in the neuronal mRNA but not in mRNA that is made in non-neuronal cells, and the use of an alternative polyadenylation site in intron 63 produces an mRNA limited to exons 1–63 that codes for a protein that lacks a carboxy (C)-terminal region. Variability in the 5' untranslated region (UTR) of the human *RELN* gene has been tentatively correlated with a genetic susceptibility to autism (BOX 1).

The Reln protein is 3461 amino acids long². As schematized in FIG. 4, the sequence begins with a signal peptide, followed by a region with similarity to F-spondin, a unique region and then eight repeats, each of around 350 amino acids. Each repeat contains an epidermal growth factor (EGF) motif at the centre, flanked by two subrepeats, A and B, which show weak similarity to one another. The protein terminates with a basic stretch of 33 amino acids, which are absent from the Reln that is coded by alternatively polyadenylated transcripts. Although the predicted molecular mass of the Reln polypeptide is 387,497 Da, the estimated size

TATA BOX
A DNA sequence with the consensus TATAAAA that is present in many eukaryotic gene promoters and specifies the site where transcription is initiated.

5'RACE
(5' rapid amplification of cDNA ends). RACE is a PCR-based method for amplifying unknown cDNA sequences by using primers that correspond to a known sequence.

ALTERNATIVE SPLICING
During splicing, introns are excised from RNA after transcription and the cut ends are rejoined to form a continuous message. Alternative splicing allows the production of different messages from the same DNA molecule.

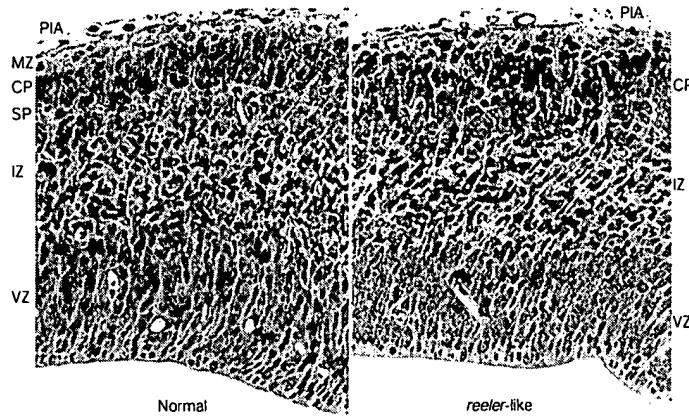


Figure 2 | Photomicrography of the normal and *reeler* telencephalon at embryonic day 14.5. 2 μm thick plastic section, stained with toluidine blue. Note the poor organization of the cortical plate (CP) and absence of a subplate (SP) in the *reeler* mouse cortex. IZ, intermediate zone; MZ, marginal zone; PIA, pial surface; VZ, ventricular zone.

METALLOPROTEINASE
A proteolase that has a metal ion at its active site.

β-PROPELLER
A protein domain that consists of an array of β-sheet motifs, which are configured in a ring to resemble the blades of a propeller.

in polyacrylamide gel electrophoresis (PAGE) is around 427 kDa (M. Sikorska, personal communication), and this ~40 kDa difference might indicate that the protein is glycosylated²¹. A similar difference is found when mammalian cells express a construct that codes for the amino (N)-terminal part of Reelin, up to the end of repeat 2. This results in the production of a 180 kDa protein, although the predicted mass is 136,107 Da. This indicates that some glycosylation might reside in the N-terminal region, but so far this has not been thoroughly investigated.

Box 1 | Reelin and susceptibility to psychoses

In addition to the well-established function of Reelin (Reelin) during brain development, some studies point to a possible role for the *Reelin* gene in conditions such as schizophrenia^{89–92} and autism^{93–98}. The human *RELN* 5'UTR contains a low and variable number of CGG repeats, and this has been tentatively correlated with genetic susceptibility to autism⁹⁷. This association should be regarded as preliminary, however, as it was not confirmed by other studies⁹⁸.

In brain samples from patients with chronic psychosis, Reelin protein and mRNA levels were reduced by approximately 50%. There were no changes in the levels of disabled 1 (Dab1) mRNA and protein; this is unexpected, as a marked upregulation of Dab1 protein levels is observed in Reelin- and lipoprotein-receptor-deficient mice. The proximal Reelin promoter is CG-rich, and like other such promoters, methylation is associated with decreased expression. Therefore, it was proposed that hypermethylation of the promoter might be the origin of decreased expression in psychiatric patients^{23–25}. Promoter methylation is known to influence long-term rather than dynamic regulation of expression, so this hypothesis needs to be investigated further.

Reelin mRNA is expressed in a subset of GABA (γ-aminobutyric acid)-expressing cortical interneurons, indicating that downregulation of *Reelin* expression might disturb the delicate balance of cortical excitability, although Reelin-deficient mice have no overt epilepsy. Heterozygous *reeler* mice have a 50% reduction in Reelin levels, and have some behavioural features that are evocative of human psychosis⁹⁹. Given the difficulty of behavioural studies in mice, these results should be independently confirmed. Taken together, these findings can be tentatively interpreted within a neurodevelopmental/vulnerability 'multi-hit' model of schizophrenia¹⁰⁰.

Reelin can be studied using antibodies against N- and C-terminal epitopes^{29,30} (FIG. 4). The full-length protein predominates in the supernatant of transfected cells, but several other fragments are also consistently found. N-terminal antibodies disclose two fragments of around 180 and 320 kDa in length, whereas C-terminal antibodies disclose fragments of around 100 and 240 kDa. In adult and embryonic brain extracts and body fluids (such as cerebrospinal fluid or plasma¹⁰), almost no full-length Reelin is detected, and the main polypeptides are the N-terminal 180 kDa and the C-terminal 100 kDa fragments (N. Ignatova and A. G., unpublished data). Comparison with partial recombinant Reelin constructs shows that Reelin is cleaved at two main locations — between repeats 2 and 3, and between repeats 6 and 7 (arrows in FIG 4). Unfortunately, there are no antibodies available to probe the central region. In embryonic brain explant cultures, Reelin processing was blocked by zinc chelators but not by other proteinase inhibitors, implying that METALLOPROTEINASE activity is involved³¹. On the other hand, Reelin cleavage is observed in different settings, implying that the processing pattern might reflect access of proteinases to exposed loops of the protein, whereas folding stabilizes other domains. The question of processing is not trivial, as the central region of Reelin is essential for receptor binding and to trigger Dab1 phosphorylation (see later discussion).

Lipoprotein receptors: *VLDLR* and *ApoER2*. The *VLDLR* gene maps to mouse chromosome 19 and human chromosome 9p24. *ApoER2*, which is also named *LRP8*, maps to mouse chromosome 4 and human chromosome 1p34. The structure and biology of these receptors have been discussed elsewhere^{32,33}, and only the features that are relevant to Reelin signalling will be summarized here. The *VLDLR* and *ApoER2* genes and proteins are similar to the low density lipoprotein receptor (*LDLR*) gene, although they are more similar to one another than to *LDLR*.

All three receptor genes code for proteins with similar organizations, but only *VLDLR* and *ApoER2* bind Reelin. Mice with inactivation of *VLDLR* and *ApoER2* have a *reeler-like* phenotype¹¹. Their structure is schematized in FIG. 5. The extracellular ligand binding region contains 7 or 8 cysteine-rich repeats (leucine-alanine (LA) repeats) of around 40 amino acids, each with three disulphide bonds and a coordinated Ca²⁺ ion. This is followed by two EGF repeats, a β-PROPELLER segment with YWTD motifs (leucine-tyrosine (LY) repeats)³⁴, another EGF repeat, a paramembrane region of O-linked glycosylation, the transmembrane segment, and a cytoplasmic region of around 60 amino acids with an NPxY sequence (where x is any amino acid). In *ApoER2*, alternative splicing can result in an insertion of 59 residues in this region, and this sequence serves as a docking site for Jun N-terminal kinase (JNK)-interacting proteins³⁵. The cytoplasmic NPxY sequence is the docking site for the protein interaction (PI)/phosphotyrosine binding (PTB) domain of the Dab1 adapter. In contrast to other PTB domain proteins, such as Shc, Dab1 binds with higher affinity to unphosphorylated NPxY sequences³⁶.

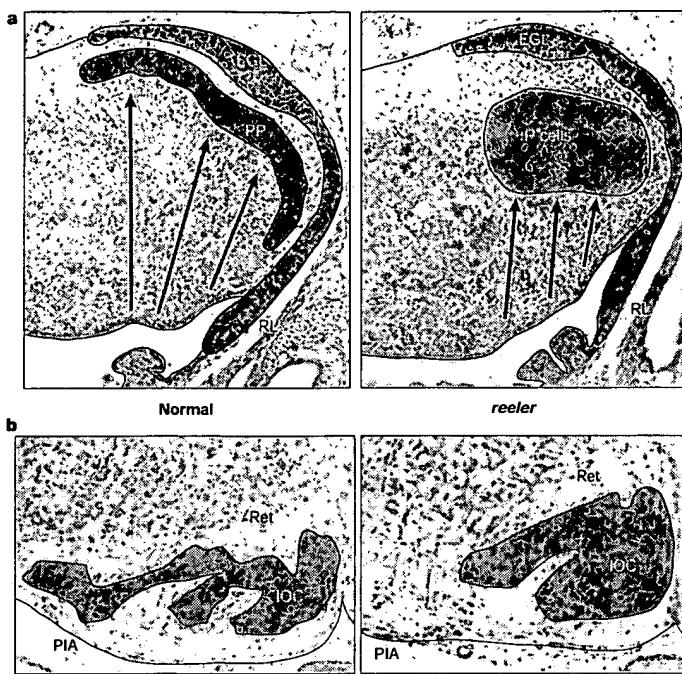


Figure 3 | The *reeler* phenotype in other brain regions. **a** | In the embryonic cerebellum, shown here at embryonic day 15.5, the *reeler* trait manifests as a poor organization of the Purkinje cell plate (PP). The external granular layer (EGL) is initially unaffected. Its inner tier contains Reelin-secreting cells, whereas Purkinje cells express Reelin receptors and disabled 1 (Dab1). The rhombic lip (RL) is where the EGL is generated. **b** | The inferior olive complex (IOC) is populated with neurons generated in the rhombic lip that reach the ventral part of the hindbrain by tangential migration. After they reach their location, normal neurons (left), which are receptor- and Dab1-positive, form defined folds, presumably under the influence of Reelin that is synthesized by surrounding neurons in the reticular (Ret.) formation. In *reeler* mice (right), olfactory neurons reach the olfactory anlage normally but fail to form a folded olive. PIA, pial surface.

CAVEOLAE
Specialized rafts that contain the protein caveolin and form a flask-shaped, cholesterol-rich invagination of the plasma membrane. They might mediate the uptake of some extracellular materials and are probably involved in cell signalling.

LIPID RAFTS
Cholesterol-rich lipid domains that are used to transport proteins around the cell and to organize signalling complexes on the membrane.

HYPOMORPHIC ALLELE
An allele that results in a reduction, but not the elimination, of wild-type levels of gene product or activity, often causing a less severe phenotype than a loss-of-function (or null) allele.

VLDLR and ApoER2 bind ligands with widely divergent structures. For example, in egg-laying species, VLDLR is known as the vitellogenin receptor, and it plays a key part in the uptake of yolk precursors³³. Despite this variety, binding of all ligands requires the LA repeat region. Furthermore, *in vitro*, all ligand binding is displaced by receptor-associated protein (RAP), an intracellular protein that prevents the premature interaction of a subset of mammalian LDLR members with ligands in the endoplasmic reticulum, and which might act as a chaperone to assist in the folding of lipoprotein receptors in the endoplasmic reticulum. In accordance with their protean roles, VLDLR and ApoER2 are not solely expressed in the brain. In mice, VLDLR is most abundant in skeletal and heart muscle, where it might participate in triglyceride uptake, whereas ApoER2 is highly expressed in the testes and placenta. LDLR is expressed in many tissues, but mostly in the liver.

Reelin can be endocytosed after binding to the surface of receptor transfected cells³⁷, but endocytosis is not required for phosphorylation of Dab1, which occurs at 4°C, a temperature that is incompatible with

endocytosis³⁸. Tyrosine phosphorylation of lipoprotein-receptor-related protein (LRP), a cousin of VLDLR and ApoER2, occurs in CAVEOLAE and involves the platelet-derived growth factor receptor- β (PDGFR β) and phosphoinositide 3-kinase³⁹. Together with the recent demonstration that ApoER2 receptors are localized to caveolae⁴⁰, these observations indicate that Reelin signalling might occur at the level of LIPID RAFTS (K. Nakajima, personal communication), although this remains to be confirmed.

Disabled 1 (Dab1). Targeted inactivation⁹ or spontaneous mutations of the *Dab1* gene, such as in the *scrambler*¹⁸ and *yotari*⁴¹ mutants, generate a *reeler*-like phenotype. Dab1 is an intracellular adaptor that is expressed in cells that respond to Reelin, such as cortical plate or Purkinje cells. Through its N-terminal PI/PTB domain, Dab1 docks to an NPxY sequence in the intracellular region of VLDLR and ApoER2 (REF. 36), and becomes phosphorylated on key Tyr residues when Reelin binds to its receptors^{42,43}.

The *Dab1* gene, which maps to mouse chromosome 4 and human chromosome 1p32-p31 (REF. 44), has a similar and surprisingly complex organization in both species⁴⁵. The coding region is spread over more than 300 kb. The main Dab1 protein (FIG. 5) is 555 amino acids long, and is encoded in 15 exons. The sequence begins with an N-terminal PTB domain of around 180 amino acids, followed by a region that contains important Tyr residues (among which Y198 and Y220 are phosphorylated in response to Reelin⁴³), and a 310 amino acid C-terminal region of unknown function. Alternative promoter use, polyadenylation and splicing generate several Dab1 isoforms. Most remarkably, the 5'UTR spreads over 900 kb, and it contains at least four different 5'UTRs and four associated promoters. One complex UTR, composed of combinations of ten different exons, is neuron-specific and developmentally regulated. Two internal protein-coding exons named 555* are consistently co-expressed in non-neuronal tissue and in stem cells in the ventricular zone, but are excluded from *Dab1* mRNA in parallel with neuronal differentiation. The expression patterns and functional implications of different Dab1 isoforms, and the origin of this highly complex genomic organization, remain to be determined.

The fact that the tyrosine phosphorylation of Dab1 is essential for signalling was elegantly shown using knock-in mice in which the endogenous locus was replaced by normal or mutant Dab1 complementary DNA (cDNA) sequences. Mice that express a cDNA in which five important Tyr residues are replaced by Phe have a *reeler*-like phenotype⁴⁶. Reciprocally, most but not all features of the *Dab1*^{-/-} mutant phenotype are rescued by replacing the *Dab1* gene by a partial *Dab1* cDNA that codes for the PTB domain and the region with the Tyr residues, thereby generating a HYPOMORPHIC ALLELE⁴⁷. So, tyrosine phosphorylation is essential but not sufficient for Dab1 function. The C-terminal region serves some unidentified function that might be related to the presence of consensus serine/threonine phosphorylation sites, some of which are substrates of the cyclin-dependent kinase 5 (Cdk5)/p35 kinase⁴⁸.

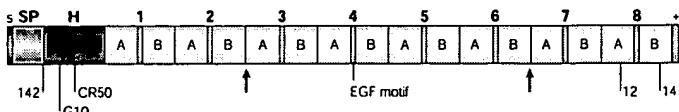


Figure 4 | Schema of the reelin protein. The sequence begins with a signal peptide of 27 residues, followed by a region with similarity to F-spondin (segment 'SP', amino acids 28–190). A unique region (segment 'H') between amino acids 191 and 500 is followed by eight repeats of about 350 amino acids (repeat 1, residues 501–860; repeat 2, 861–1220; repeat 3, 1221–1596; repeat 4, 1597–1947; repeat 5, 1948–2314; repeat 6, 2315–2661; repeat 7, 2662–3051; repeat 8, 3052–3428). Each repeat contains an epidermal growth factor (EGF) motif at the centre, flanked by two subrepeats, A and B, which show weak similarity to each other. The protein terminates with a stretch of 33 amino acids that is rich in basic residues (3429–3461, +). The epitopes recognized by antibodies 142, G10, CR50, 12 and 14 are shown, and the two arrows point approximately to the sites of processing *in vivo*. Adapted, with permission, from *Nature* REF. 2 © (1995) Macmillan Magazines Ltd. s, signal peptide.

Unravelling the network

The dissection of the Reelin signalling network is proving to be difficult, because it does not seem to conform to any known models. However, progress was recently made on two fronts — the interactions between Reelin and its receptors, and the characterization of tyrosine kinases that have been implicated in Dab1 phosphorylation.

Reelin-receptor interactions. The N-terminal moiety of Reelin does not bind to the VLDLR or ApoER2 receptors¹⁰, but nevertheless there is evidence that the N terminus contributes to signalling. The function-blocking CR50 antibody, which is directed against an N-terminal epitope, interferes with the binding of Reelin to its receptors³⁷ and inhibits aggregation of the protein — a process that is considered to be important for Reelin's functional activity^{49,50}.

To find out how Reelin interacts with its receptors, binding of partial Reelin recombinant proteins to extracellular receptor regions was studied *in vitro*. The same recombinant proteins were also assessed for their capacity to stimulate Dab1 phosphorylation in primary neuronal cultures^{42,51}. Proteins comprised of the N-terminal region, the first four repeats and part of repeat 5 do not bind significantly, confirming that the N-terminal moiety of Reelin is not directly involved^{10,15,42}. Proteins that contain one, two or three repeats fail to bind detectably to VLDLR and ApoER2. By contrast, proteins comprised of repeats 3–8 or repeats 3–6, and a Reelin mutant with its 388 N-terminal amino acids deleted, all bind to VLDLR and ApoER2 with an affinity that is comparable to full-length Reelin. The ability of recombinant proteins to bind to receptors and stimulate Dab1 phosphorylation is correlated. These experiments indicate that at least four repeats are necessary to activate the Dab1 part of the signal, and that the repeats that are contained in the central region of Reelin are particularly important. It is interesting to note that this region corresponds to the central fragment that is generated by proteolytic processing⁵¹.

These results seem to contradict the observations that the N-terminal region is important for Reelin function^{49,50,52,53}. However, this discrepancy could reflect differences between the *in vivo* and *in vitro* situations. *In vivo*, CR50-sensitive Reelin aggregation through the

HEPARAN SULPHATE
A glycosaminoglycan that consists of repeated units of hexuronic acid and glucosamine residues. It usually attaches to proteins through a xylose residue to form proteoglycans.

N terminus^{49,50} could increase the effective Reelin concentration, thereby recruiting a larger set of receptors and signalling molecules, and enhancing signal efficacy.

Dab1 tyrosine phosphorylation — the elusive kinase.

Although tyrosine phosphorylation of Dab1 is known as a key step in Reelin signalling, Fyn has only recently been identified as the main kinase that is involved in this signalling event^{54,55}. *In vitro*, the ability of the cytoplasmic tyrosine kinases Fyn, Src and Yes, as well as Abl, to phosphorylate Dab1 was shown at the time of cloning of the *Dab1* gene⁹. Fyn can be co-immunoprecipitated with Dab1, Reelin and receptors from embryonic brain extracts (Y. Jossin and A.G., unpublished data). Defective Reelin signalling is marked by an increase in Dab1 protein levels and a decreased ratio of phosphorylated to unphosphorylated Dab1 (REF. 42). Similar modifications are seen in mice with different dosages of the *Fyn*, *Src* and *Yes* genes. The data indicate that Fyn is the main Dab1 kinase *in vivo*, followed by Src and to a lesser extent by Yes, whereas Abl does not play a significant part.

How the Fyn and other cytoplasmic tyrosine kinases are recruited to the signalling complex is not known, although an appealing model has implicated protocadherins of the CNR family⁵⁶. CNR1 binds Fyn in its cytoplasmic tail and was thought to bind the N-terminal region of Reelin in its extracellular domain, thereby enabling Reelin to bring Fyn into the receptor complex. However, the binding of Reelin to CNR1 has not been confirmed. Furthermore, the observation that Dab1 phosphorylation can be stimulated by a Reelin molecule that lacks an N terminus argues against this mechanism. Recent studies^{54,55} indicate that Reelin might assemble a prepared complex that regulates the proximity of Fyn to Dab1, so that Dab1 induces Fyn activation and is phosphorylated in return. Fyn might be activated by the interaction of Dab1 with its SH3 domain, or after tyrosine phosphorylation of Dab1 with the Fyn SH2 domain. A similar model has been invoked for the tyrosine phosphorylation of the T-cell receptor by the Src-family kinase Lck, which is associated with and activated by clustering with CD4 (REF. 57).

An objection to the proposed key roles for Fyn and Src is raised by the observation that neither *Fyn*^{-/-}, *Src*^{-/-}, nor *Fyn*^{-/-}; *Src*^{-/-} double mutants, possess a *reeler*-like phenotype^{54,55}, possibly due to functional redundancy. *Fyn*^{-/-}; *Src*^{-/-} double mutants die perinatally and have not been studied. In favour of redundancy, there is preliminary evidence that a *reeler*-like malformation can be induced in brain slice cultures by PP2, a wide spectrum inhibitor of Src family kinases (Y. Jossin and A.G., unpublished).

In addition, the Reelin pathway might interact with another signalling complex (FIG 5). Proteoglycans, such as the HEPARAN SULPHATE proteoglycans and syndecans, modulate signalling by growth factor-receptor complexes⁵⁸. Although Reelin is not a proteoglycan, its large size indicates that it could have a somewhat similar role, for example by providing several binding sites and concentrating growth factors locally. Furthermore, LRP modulates the PDGFR pathway^{39,59} and another related

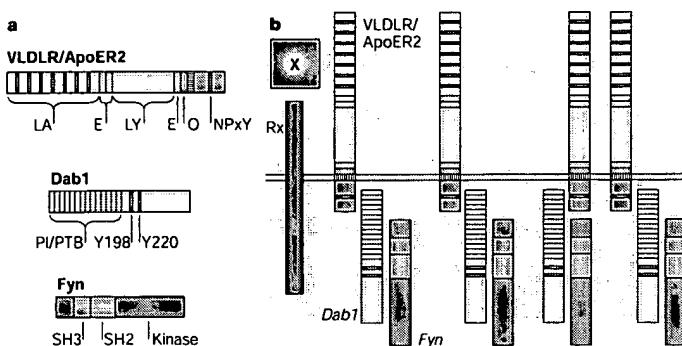


Figure 5 | The partners of reelin signalling. **a** | The very-low-density lipoprotein receptor (VLDLR) and apolipoprotein E receptor type 2 (ApoER2) extracellular ligand binding region contains 7 or 8 imperfect cysteine-rich repeats (leucine-arginine (LA) repeats, yellow) of around 40 amino acids, each with three disulphide bonds and a coordinated Ca^{2+} ion. This is followed by two epidermal growth factor (EGF) repeats (blue), a β-propeller segment (leucine-tyrosine (LY) repeats), usually made of 5 repeats of about 50 residues with a F/YWxD consensus (where x is any amino acid), another EGF repeat, a paramembrane region of O-linked glycosylation (O), the transmembrane segment, and a cytoplasmic region of about 60 amino acids with the key NPXY sequence. The main disabled 1 (Dab1) protein isoform has 555 residues and is composed of an amino-terminal PI/PTB domain of about 180 amino acids that docks to the NPXY sequence in receptors, followed by a region with Tyr residues, two of which (Y198/220) are essential for signalling. Fyn family kinases have one SH3, one SH2 and a kinase domain as shown. **b** | The central part of reelin docks to a complex of lipoprotein receptors and Dab1, and tyrosine kinases of the Fyn group play a key part in signal transmission. There is a possibility that another ligand (denoted by 'X') and corresponding receptor ('Rx'), would interact with the reelin signal.

molecule, LRP6, is a key regulator of Wnt signalling⁶⁰. The failure to identify such a 'missing component' among the many spontaneous or induced mouse mutants could be due to embryonic lethality or redundancy. This view is admittedly highly speculative, and the identity of this putative signalling system, which might be coupled to Reelin, remains open to debate.

The action of Reelin in the developing brain

The observations summarized earlier leave the question of what Reelin is actually doing in the developing brain relatively untouched. Although our view is still fuzzy, we will discuss recent observations that point to a possible role for Reelin in radial precursor cells, before ending with some speculations on the cellular action of Reelin.

Reelin and neural precursor cells. In man and rodents, *Dab1* mRNA, as well as mRNAs for VLDLR and the ApoER2 receptor, are expressed in the ventricular zone — presumably in neuronal precursor cells. The Dab1 protein is detected in the same cells as *Dab1* mRNA, but data on the VLDLR and ApoER2 proteins are sketchy^{45,61}. The relative intensity of *VLDLR* and *ApoER2* mRNA hybridization in the ventricular zone differs among brain areas, as well as between developmental stages. These preliminary results indicate that precursor cells possess the machinery to respond to Reelin. One target of Reelin in the ventricular zone could be the future olfactory interneurons that follow the rostral migratory stream. Interestingly, it was shown that Reelin facilitates the detachment of these interneurons from the stream, and promotes their

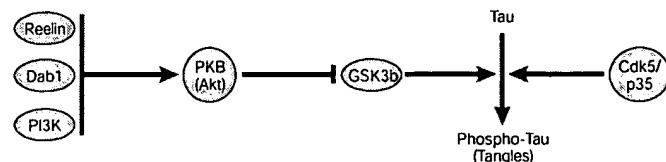
migration to the olfactory bulb⁶². Expression of Dab1 and Reelin receptors in the ventricular zone agrees with the proposed role for Reelin in the maturation or migratory capacity of neural stem cells⁶³. Reelin assists in the formation of the radial glial scaffold in the dentate gyrus and promotes branching of radial glial cells *in vitro* through a Dab1-dependent mechanism⁶⁴, further pointing to a role for Reelin signalling in radial precursor cells.

Unlike other species such as chick and crocodile⁶⁵, no Reelin-positive cells are detected in the vicinity of the telencephalic ventricular zone in mammals, raising the question of the origin of the ligand that binds to the receptors in precursor cells. Reelin secreted from Cajal–Retzius cells could bind to lipoprotein receptors on the radial processes of precursor cells that expand through the whole thickness of the tissue. The observation that Reelin promotes branching of these radial processes⁶⁴ concurs with this view, which also predicts that the VLDLR and/or ApoER2 protein(s) should be present on radial processes in the marginal zone, close to Reelin-producing cells. Alternatively, Reelin or its active central fragment could diffuse from the marginal zone to the ventricular zone. In principle, these ideas should be easy to check. However, so far, it has proved difficult to detect extracellular Reelin reliably. In addition, antibodies are not yet available to allow immunohistochemical studies of the central fragment of Reelin and the lipoprotein receptors.

Given the potential links between Reelin signalling, psychoses and Alzheimer's disease (BOXES 1 and 2), the effect of this pathway on neural stem cells is potentially important, especially if it continues to operate in the adult brain and influence regeneration, as indicated by the observation that decreased Reelin expression seems to influence granule cell dispersion in epilepsy⁶⁶.

Cell biological mechanisms of Reelin action. Reelin is present in the nervous system in all vertebrates, from lamprey⁶⁷, and even *Amphioxus* (G. Meyer, personal communication), to fish, *Xenopus*, reptiles, birds and mammals^{26,65,68}, and its expression is widespread. Comparative studies of Reelin expression in the embryonic cortex indicate that Reelin-positive Cajal–Retzius cells are present in the marginal zone in all amniotes, indicating that these cells are evolutionarily homologous. However, mammalian Cajal–Retzius cells are characterized by a striking amplification of Reelin production, pointing to the spatiotemporal control of Reelin expression as a key feature of cortical evolution^{26,65}. Like Cajal–Retzius cells, cerebellar granule cells synthesize large amounts of Reelin in all species, and this is consistent with the cerebellar malformation that is observed in Reelin-deficient mice and humans. On the other hand, the *reeler* malformation is subtle, and until recently had not been detected in many areas where Reelin expression is now known to be strong, such as the retina⁶⁹ and spinal cord¹³. The same contrast between high Reelin expression and almost no alterations in *reeler* mice is even seen in some laminar structures, such as olfactory mitral cells. However, the parsimony principle dictates that a single molecular mechanism should explain all observations, until proven otherwise.

Box 2 | Reelin and tau phosphorylation: a link with Alzheimer's disease?



Alzheimer's disease (AD) is the leading cause of dementia in ageing populations. It is characterized by progressive brain degeneration and two key pathological findings: extracellular amyloid plaques composed of the amyloid peptide A4 — a processing product from the amyloid precursor protein (APP) — and the presence of intraneuronal tangles made of a hyperphosphorylated form of the tau microtubule-associated protein. Hereditary forms of AD are due to mutations in APP or presenilin 1 and 2 — two cofactors of APP proteolytic processing. Non-hereditary forms of AD are more frequent in carriers of the E4 allele of the apolipoprotein E (*ApoE*) gene.

Several intriguing putative links have been noted between AD and the reelin (Reelin) pathway. First, *ApoE* is present in the brain and is a ligand for Reelin receptors, and it might modulate Reelin signalling¹⁰¹. Second, APP and disabled 1 (Dab1) interact in YEAST TWO-HYBRID SCREENS³⁶, and Dab1 binds to the cytoplasmic tail of APP¹⁰². Third, Cdk5 (REF. 103), in complex with its cofactors p35 and p39 (REF. 104), is a key tau kinase that can phosphorylate Dab1 (REF. 48). Cdk5 is essential for neuronal migration and has been implicated in AD¹⁰⁵. Fourth, defective Reelin signalling is associated with increased tau phosphorylation¹⁰. Conversely, activation of the signal results in a reduction of tau phosphorylation, and this is due to reduced kinase rather than increased phosphatase activity^{101,106}. Another intriguing finding is that increased tau phosphorylation in mice with defective Reelin signalling is under the influence of modifier genes, one of which maps to chromosome 17 in the vicinity of the APP locus¹⁰⁷. Recent results (see figure) showed that, among the main tau kinases, Cdk5 activity is unaffected by Reelin, whereas glycogen synthase kinase 3β (GSK-3β) is inhibited by Reelin through activation of protein kinase B (PKB)¹⁰⁶. Reelin activates PKB by phosphorylation at S473, one of two sites that are crucial for activation (the other is T308). The activation of PKB inhibits the activity of GSK3β by phosphorylation at S9. The Reelin-induced phosphorylation of PKB on S473 requires activation of phosphatidylinositol 3-kinase (PI3K), and is Dab1-dependent. Although it is impossible to present an integrated view at this stage, these observations strongly imply that the Reelin pathway has a role in AD pathogenesis.

YEAST TWO-HYBRID SCREENS
System used to determine the existence of direct interactions between proteins. It involves the use of plasmids that encode two hybrid proteins; for example, one of them is fused to the GAL4 DNA-binding domain and the other one is fused to the GAL4 activation domain. The two proteins are expressed together in yeast and, if they interact, then the resulting complex will drive the expression of a reporter gene, commonly β-galactosidase.

SOMAL TRANSLOCATION
Displacement of the cell body, as opposed to migration of the whole cell.

BLASTOCYST INJECTION
The introduction of embryonic stem cells into a blastocyst-stage embryo of a different genotype to generate a chimaeric embryo.

In the *reeler* cortex, cortical plate cells keep a prolonged contact with radial glial guides and invade the marginal zone to pile up close to the pia. This led to the proposal that Reelin could provide a 'stop' signal to neurons at the end of their migration pathway^{70,71}. Like the migration of other cells, neuronal migration proceeds by the extension of a leading edge, followed by progression of the nucleus in this cytoplasmic furrow (nucleokinesis)⁷². Leading edge extension requires actin polymerization/depolymerization, with formation of filopodia and lamellipodia in some cells, followed by consolidation of stress fibres. These processes are controlled by the small GTPases Cdc42 (filopodia formation), Rac (lamellipodia formation) and Rho (consolidation of stress fibres). Nucleokinesis depends on microtubule dynamics, in addition to microfilaments.

Recent findings on radial neuronal migration have introduced an additional level of complexity into the 'stop signal' model. The new data indicate that radial neuroepithelial or glial cells are direct neuronal precursors⁷³⁻⁷⁵ and that early migrating neurons might use SOMAL TRANSLOCATION rather than gliophilic migration^{76,77}. The latter mode of radial migration seems to become

more important at later stages, when migration distances increase.

Clearly, the idea of a 'stop' signal requires formulation in cell biological terms. Although *reeler* mice have abnormal entorhinal-hippocampal connections^{78,79}, this is probably secondary to architectonic disturbances, as Reelin does not seem to influence leading edge extension or growth cone progression^{80,81}. However, Reelin could negatively regulate nucleokinesis, and influence the position of the nucleus relative to processes and ramifications⁸². The idea that Reelin might act as an inhibitor of nucleokinesis could account for several observations. For example, in the tectum and cochlear nuclei, inhibition of the progression of neuronal nuclei by Reelin might explain why, in *reeler* mutants, abnormally orientated neurons invade a cell-poor subpial zone. In the *reeler* spinal cord, spinal presynaptic neurons are in an ectopic position, close to the central canal^{13,83}. However, the reason is not a failure to migrate, but rather a late back-movement from outside to inside. This back-movement does not occur in normal animals, in which a Reelin-positive layer is found deep in migrated presynaptic neurons, implying that Reelin inhibits this back nuclear translation. In the *reeler* inferior olive, the long tangential migration from the rhombic lip occurs normally, but neurons seem to migrate too far, too close to the midline. This indicates that Reelin secreted by the reticular formation might govern the location of the olfactory neuronal nucleus and soma in relation to their processes.

On the other hand, some aspects of the *reeler* phenotype do not fit easily with this action on nucleokinesis. In the hippocampal formation, the *reeler* trait consists mostly of a poor lamination of the pyramidal cell layer and dentate granule cell layer, but this is due to failure of neurons to reach their respective layers rather than invasion of the marginal zone. In the *reeler* cerebellum, radially migrating Purkinje cells stop migration prematurely and fail to condense into a normal Purkinje cell plate. The defect in the molecular layer is due to the secondary degeneration of granule cells and not to over-migration of Purkinje cells. Similarly, in the *reeler* facial nerve nucleus, ectopic neurons settle in the tegmentum, and there is little or no overmigration towards the pia. The subtle malformations in the *reeler* retina are also not obviously explained by an effect on nucleokinesis.

Furthermore, an action of Reelin as a direct negative regulator of nucleokinesis is difficult to reconcile with two important sets of observations. First, expression of the *Reelin* cDNA under the control of the nestin promoter, which results in ectopic Reelin expression in precursor cells in the ventricular zone, can partially but significantly rescue the *reeler* phenotype⁸⁴. It is difficult to imagine how Reelin, in the ventricular zone, could directly influence the terminal migration of neurons that have already left it. However, it raises the possibility that Reelin could make neurons responsive to an unidentified signal in the marginal zone.

A second question has arisen from studies of normal and *Dab1*^{-/-} chimaeric mice^{85,86}. In the cerebral cortex of chimaeras produced by BLASTOCYST INJECTION, *Dab1*^{+/+} cells

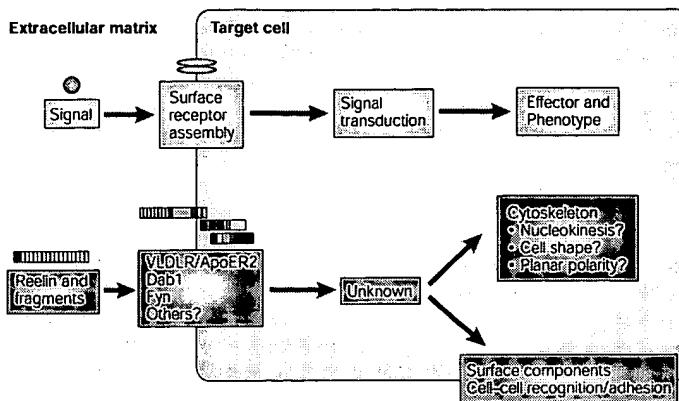


Figure 6 | Putative mechanism of action of Reelin. Only the proximal end of the reelin signalling pathway (FIG. 4) is known at present. Signal transduction is poorly understood, and the final effect of signal activation on target cells is also not clear. As explained in the text, reelin could influence the cytoskeleton and regulate nucleokinesis, as well as modulate expression of cell-cell interaction molecules on the cell surface. ApoER2, apolipoprotein E receptor type 2; Dab1, disabled 1; VLDLR, very-low-density lipoprotein receptor.

MORULA AGGREGATION
A technique in which the cells of two different embryos at the morula stage (when the embryo comprises a solid ball of cells) are dissociated, mixed and allowed to recombine to generate a chimaeric embryo.

segregate from *Dab1*^{-/-} cells, thereby forming two superposed cortical plates. Although this supports a cell-autonomous function for Dab1, a few normal cells were consistently located in an ectopic position in the abnormal cortex⁸⁵. Similarly, in the cerebellum and hippocampus of chimaeric mice produced by MORULA AGGREGATION⁸⁶, a few mutant Purkinje and pyramidal neurons were found in ectopic positions. If Reelin directly determines the position of the nucleus, it would be predicted that all of the ectopically positioned cells would be deficient in Dab1. These observations imply that the response to Reelin requires collaboration among several target cells — a 'community effect'⁸⁶ — and points to the importance of surface recognition molecules, as already proposed long ago⁸⁷ and emphasized

again recently⁸⁸. One could imagine a Reelin signalling network that provides instructions to migrating cells by modifying the synthesis of some surface proteins and/or directing them to specific cellular compartments (FIG. 6). Such a signal would be indirect, with a delay due to signal transduction and response of the target cell. This response is probably complex and might involve cytoskeletal components which regulate nucleokinesis, the upregulation or downregulation of cell adhesion molecules^{62,88}, the routing of surface recognition components to specific surface domains, and probably other unanticipated elements.

Future prospects

To conclude, we would like to propose two broad questions that we consider to be important, and that should be amenable to analysis with the available technology. First, we need to understand better the factors that regulate Reelin expression, particularly in cortical Cajal-Retzius neurons. This will provide a molecular insight into the complex problem of cortical evolution. The ever-increasing availability of genomic sequences allows the definition of conserved DNA segments in homologous genes — for example, Reelin and its partners — in different species. Presumably, such evolutionarily conserved sequences contain regulatory elements that could then be tested by gene electroporation *in vivo* or *in vitro*, and in transgenic mice.

Second, we should try to define the modifications in synthesis and/or distribution of cell-cell interaction proteins that result from Reelin signalling. Progress in cDNA subtraction techniques and cDNA microarray screening should allow the definition of some genes that are downregulated or upregulated by Reelin signalling. Better *in vitro* models for radial neuronal migration could be developed to study the role of different signalling pathways that control this delicate process and also to interfere with them. We can only guess what the answers to these key questions will turn out to be, but they will undoubtedly be exciting, and most probably unexpected.

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Acknowledgements

We wish to thank C. Lambert de Rouvroit for her help in the preparation of this review, as well as I. Bar, Y. Jossin and N. Ignatova for discussions and inclusion of unpublished results. We apologize to colleagues for the inevitable selection of citations due to space limitations. This work was supported by the Fondation Médicale Reine Elisabeth, and by the fifth framework program of the European Union.

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